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INVENTOR(S)		
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Additional inventors are being named on the _____ separately numbered sheets attached hereto

TITLE OF THE INVENTION (500 characters max)

Method for Cultivating Edible Nostoc commune

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ENCLOSED APPLICATION PARTS *(check all that apply)*

☒ Specification *Number of Pages* 3 ☐ CD(s), Number _____

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Respectfully submitted,

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Method For Cultivating Edible *Nostoc commune*

Abstract

This invention provides a method for cultivating *Nostoc commune* (also known as *Nostoc sphaericum*, or *Nostoc commune var. sphaericum*), comprising (a). steps of isolating and purifying the said organism; (b). steps of culturing the said organism; and (c). conditions suitable for optimal growth of the said organism.

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References Cited

Aki B., Heilmann J. and Sticher O. (2000) New antibacterial metabolites from the cyanobacterium *Nostoc commune* (EAWAG 122b). J Nat. Prod. 63: 1283-1285.

Brown M.R. (1991) The amino acid and sugar composition of 16 microalgae used in mariculture. J. Exp. Mar. Biol. Ecol. 145: 79-99.

Brown M.R. and Jeffery S.W. (1995) The amino acid and gross composition of marine diatoms potentially useful for mariculture. J. Appl. Phycol. 7: 521-527.

Hayashi, K. Hayashi, T. & Kojima, I. 1996. AIDS res. Human Retroviruses 12: 1463-71.

Hu Q., Guterman H. and Richmond A. (1996) A flat inclined modular photobioreactor (FIMP) for outdoor mass cultivation of photoautotrophs. Biotechnol. Bioeng. 51: 51-60.

Komarek J. and Anagnostidis K. (1989) Modern approach to the classification system of cyanophytes. 4-Nostocales. Arch. Hydrobiol. Suppl. 823, Algological Studies 56: 247-345.

M. K. Ang-Lee, J. Moss, and C.S. Yuan (2001) Herbal medicines and perioperative care. JAMA 286: 208-216.

Philippis R.D. and M.Vincenzini (1998). Exocellular polysaccharides from cyanobacteria and their possible applications. FEMS Microbiol. Rev. 22: 151-175.

Piccardi R., Frosini A., Tredici M.R., Margheri M.C. (2000). Bioactivity in free-living and symbiotic cyanobacteria of the genus *Nostoc*. J Appl. Phycol. 12: 543-547.

Scherer S. and Potts M. (1989) Novel water stress protein from a desiccation-tolerant cyanobacterium: purification and partial characterization. J. Biol. Chem. 264: 12546-12553.

Wolk C.P. (1996). Heterocyst formation. Ann. Rev. Genet. 30: 59-78.

Description

BACKGROUND OF THE INVENTION

The cyanobacterium *Nostoc commune*, also known as *Nostoc sphaericum*, or *Nostoc commune var. sphaericum*, is a filamentous, nitrogen-fixing cyanobacterium belonging to the family of *Nostocaceae* in the order of *Nostocales* (Komarek and Anagnostidis, 1989). In natural habitats, such as rice paddies, shallow streams, water pounds, and large open fields, *N. commune* can form spherical macroscopic colonies consisting of filaments embedded in a gelatinous matrix. The size of colonies ranges from tens of mm to tens of cm in diameter with the largest described being 2.6 kg wet weight (Dodds et al. 1995). The colonies range in colors from yellow-green to red-brown, and dark green to black (Potts, 2000). The filaments are unbranched and largely twisted, and consist of mostly vegetative cells with a few heterocysts occurring in the middle of a filament. Reproduction of *N. commune* takes place in four different ways, depending on environmental conditions: 1) single cells of *N. commune* fragmented from filaments can form new colonies; 2) akinete formation and germination; 3) hormogonia disperse and form new colonies; 4) large colonies can bud off to form separate colonies (Dodds, et al., 1995).

SUMMARY OF THE INVENTION

The present invention relates to *Nostoc commune* cells, a *Nostoc commune* strain and methods for mass cultivation of *Nostoc commune*. More specifically, this invention relates to a methodology used for cultivating *Nostoc commnue*. This invention further relates to methods for large-scale cultivating *Nostoc commune*.

DETAILED DESCRIPTION OF THE INVENTION

Section 1. Methodology for strain isolation and purification

Colonies of *Nostoc commune* were collected from Yadkin River, Forsyth County, North Carolina during the spring. After washing with sterile Algaen-I medium, colonies was crashed with pestle and mortar, cells were spread onto the Algaen-I agar plates. The plates were illuminated with fluorescent light. After one week, cells from the plates were transferred to a fresh plate. After three transfers, axenic colonies were obtained, which were used for further cultivation.

Section 2. Steps for cultivating *Nostoc commune*:

The cultivation of *Nostoc commune* consists of the following steps:

3a. Hormogonia generation in Algaen-II

3b. Formation of micrcolonies on agar plate containing Algaen-III medium

3c. Growth and reproduction of microcolonies in culture vessels

3d. Formation of macrocoloies in culture vessels.

Section 3. Optimal conditions for each cultivating steps described above.

4a. Hormogonia generation:

To induce hormogonia generation, macrocolonies of *Nostoc commune* were washed three times with Algaen-II medium as described in section 2. The washed macrocolonies were re-suspended in the said Algaen-II medium for 3 days at 25°C with illumination of 100 μmol photon·m^{-2}·s^{-1}, hormogonia were released from the colonies.

Hormogonia may be obtained also by grinding the macrocolonies with pestle and mortar in Algaen-I medium.

4b. Formation of microcolonies on agar plates:

Hormogonia were resuspended in Algaen-I medium and spread on agar plate containing 1.5% agar and Algaen-I medium at concentration of 1000 cells/plate. The plates were sealed with parafilm and incubated at 25°C with illumination of 10 μmol photon·m^{-2}·s^{-2}. After one week, formation of microcolonies was observed with microscope. After three weeks, the microcolonies were ready for transfer to liquid growth medium.

4c. Growth and reproduction of microcolonies in culture vessels

The microcolonies obtained from agar plates mentioned above were transferred to a culturing vessel containing 200 ml l Algaen-I medium. The culture was illuminated with fluorescent bulbs at light intensity of 200 μmol photon·m^{-2}·s^{-2}. Mixing of the culture was provided by bubbling the liquid with CO_2-enriched air. The culturing vessels can be glass bottles, transparent plastic bottles or other transparent containers. The volume of the culturing vessels can be at a range of 100 ml to 20 liter.

4d. Formation of macrocolonies in culturing vessels

To induce the formation of macrocolonies, microcolonies were transferred to the following conditions: (a). Algaen-III medium as described in Section 2; (b). Light intensity of 500 μmol photon·m^{-2}·s^{-1}. Under these conditions, microcolonies stopped division and reproduction, instead, all microcolonies continuously grew to increase their volume. The diameter increased from less than 1 mm to more than 3 mm after one week. After two weeks, 90% of colonies reached diameter of 5 mm. After four weeks, about 80% colonies reached diameter of 10 mm.